

amino acid and a faint hexoseamine spot were evident at 24 hours. On the other hand, the HCl treatment gave strong glucose spots at both 5 and 24 hours. It was apparent that readily soluble substances detected in the younger culture were no longer present in older cells but had been converted to a storage material yielding glucose upon acid hydrolysis. This storage material tended to decrease in amount as the cells aged.

Sludge Glycogen

Many microorganisms contain glycogen as a storage carbohydrate much as it is found in muscle and other animal tissue. Edible fungi contained 12.6 per cent ¹¹⁸ while a filamentous form had 36.7 per cent glycogen. ²⁶ Glycogen content of yeast depended upon treatment, ¹⁶ while bacteria may have as high as 50 per cent. ⁸¹

Glycogen determination of the sludge showed 19.3 per cent in the 5-hour sample and 8.8 per cent in the 24-hour sample. Another sludge maintained unfed at 30°C for 24 hours contained only 2.9 per cent of the glycogen-like fraction.

That glycogen-like products are stored may be inferred from the data in Table 1-14 when the COD of the sludge is considered. A 1000-ppm sludge has a COD of 1250 ppm. At 4 hours, 27.9 per cent of the 1172 ppm skim milk COD was in new cells; hence, the total cell COD would be 327 plus 1250 or 1577 ppm. The stored COD was 49.9 per cent of the skim milk COD, or 585 ppm. This latter value is calculated as 27 per cent of the total sludge COD and approaches the 19 per cent glycogen fraction, especially if we consider that in 5 hours synthesis would be greater and storage decreased.

It becomes apparent that the rapid purification of a carbohydrate waste results from more involved changes than simple adsorption. A portion of the COD is changed to CO_2 , another portion is synthesized into cell complex with a low immediate oxygen demand, while the remainder is converted and stored as insoluble glycogen-like substance. The oxygen demand of the mixed sludge continues at a high rate while the storage carbohydrate is oxidized and then drops to that of true endogenous respiration.

1-4 FACTORS AFFECTING EFFICIENCY AND SOLIDS PRODUCTION IN THE ACTIVATED SLUDGE PROCESS

049

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This paper discusses the influence of several factors on activated sludge performance. All the discussions are predicated on the assumption that the wastes treated are nutritionally balanced.

The great diversity of organisms composing activated sludge is best demonstrated by the large number of pure, organic substances which are respired by normal sludge without any adaptation period. This has been shown in manometric experiments of Dawson and Jenkins.²¹ Complex media, such as domestic sewage, is also respired without any observable lag period. ¹⁶² It has not been possible to date to demonstrate intermediate degradation products when nutritionally balanced substrates were adequately aerated. The observable over-all reaction of a waste with sludge is considered the resultant of many single reaction chains. Activated sludge is considered as a whole when the stabilization of organic substances and their intermediate metabolic products are studied.

The two parameters of primary importance in process evaluation are the quantity of oxygen required and the detention period necessary for a specified purification level.

The quantity of oxygen which will be consumed may be evaluated by manometric measurement of the endogenous and substrate respiration in contact with sludge. Simultaneous observation of the concentration change of the substrate during the respiration experiment permits the calculation of the ratio of substrate respiration to assimilation. A portion of the organic substrate removed by activated sludge is used for new cell synthesis and storage products and consumes no oxygen. The remainder undergoes direct oxidation.

Figure 1-27 shows observations in an experiment where saccharose in phosphate buffer was added to sludge which was not previously adapted to saccharose. This experiment leads to the following conclusions:

(a) Saccharose is respired with a respiration quotient (R.Q.) of 1, while

industrial wastes were greater than the rates of oxidation. These rates varied with the type of waste and other factors, but averaged about four times the rate of oxidation. For dairy waste they found a rate of purification six times the rate of oxidation under their conditions. In a mechanically stirred aerator, higher rates have been attained by the authors using 1000 ppm skim milk and 1000 ppm sludge solids. The relative rate of purification averaged about twelve times the rate of oxidation. Almost 80 per cent of skim milk COD was removed from solution in 1 hour. During the same time the oxygen utilized, as measured in the Warburg respirometer, was equivalent to only 50 per cent of the COD. The difference must be stored material. In 90 minutes all the skim milk had been removed from solution, but oxygen utilization continued at the same rate for another 90 minutes. Evidently the stored material was being oxidized. Previous Warburg studies showed that about 6 hours were required by 500 ppm sludge solids to oxidize 1000 ppm milk solids; hence 1000 ppm sludge should require about 3 hours.

According to the equations for the synthesis of cell material from skim milk, 37.5 per cent of the COD is completely oxidized in the removal of 100 per cent from solution. Therefore, it seems reasonable to consider that the over-all rate constant for the removal of nutrients should be 2.67 times that of the oxidation constant if there is no storage of material. Higher rate constants indicate a nonoxidative accumulation of nutrients in the organisms that undergo a subsequent rapid oxidation. This is followed by endogenous respiration.

Studies on oxidation, purification, and storage were continued under less vigorous agitation using quart jar aerators. Lach jar, holding 650 ml of a mixture containing 1172 ppm skim milk QOD and 1000 ppm sludge solids, was aerated with CO₂-free air at the rate of 325 ml per min. Even though there was no vigorous agitation, removal of COD from the solution was rapid, about 95 per cent in 4 hours at 30°C (Table 1-14). Considerable purification occurred also at lower temperatures; 89 per cent at 20°C; 67 per cent at 10°C; and 45 per cent at 2°C.

The COD removed in cell formation is the amount oxidized plus that used for cell synthesis and is the theoretical amount removed during the

TABLE 1-14. COD REMOVED IN PURIFICATION AND AMOUNTS USED FOR RESPIRATION, SYNTHESIS, AND STORAGE

(1000 ppm studge solids and 1172 ppm skim mil	k COD)
Time (hr)	Purification Respiration Synthesi (%) (%) . (%)	Storage (%)
1	61.6 5.2 8.6	47.8
2	85.6 9.4 15.6	60.6
3	90.8 13.1 21.9	55.8
4	94.6 16.8 27.9	49.9

assimilation step. Thus the values from Curve C in Figure 1-26 times 2.67 give Curve D. Curve B shows the actual removal of COD; hence, the difference between B and D must be unassimilated, or stored. Curve A is removal of lactose. In the jar experiments (Table 1-14) as much as 60 per cent of the available COD was stored. In less than 20 hours, the stored material was gone and oxidation of the cells was under way.¹¹⁵

Determination of the type and site of this unassimilated stored material required larger quantities of cell material. Sludge was prepared at 30°C in the large aerator and then held at 10°C, since stored material metabolizes quite slowly at that temperature. Agitation and aeration were continued after addition of skim milk. After 5 hours and 24 hours, 6-liter quantities of the mixture were removed. Sludge was harvested by centrifuging, washed with saline solution, recentrifuged, and lyophilized.

Paper Chromatography

Half a gram of cell material was treated in a boiling water bath successively with 25 ml hot water and 50 per cent alcohol for 1 hour and for 2 hours with 2 per cent HCl. Extracts were recovered by centrifuging and papergram analyses for sugars were made. 116

Lactose was absent in all sludge samples. The trace of glucose present in the 5-hour hot-water sample did not appear in the 24-hour sample. Also most of the glucose and all of the maltose observed at 5 hours in the 50 per cent alcohol solution was gone in 24 hours. The alcohol solution showed the presence of four amino acids and a hexoseamine at 5 hours. Only one

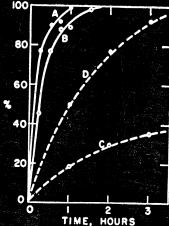


FIGURE 1-26. Purification, oxidation, and synthesis by 1000 ppm aerator sludge (substrate, 1000 ppm skim milk). A = removal of lactose; B = removal of COD; C = oxygen uptake or respired; D = COD synthesized and respired (oxygen uptake ×2.67).

An almost exact equivalent of the amount of oxygen required for the assimilation of lactose and casein in the manometric studies is explained by this series of equations.

Skim Milk Conversion to Sludge Cells

A comparison of the assimilation equations for lactose and case in showed that a mole of cell substance was produced from 240 units of sugar and from 184 units of case in. This is practically the same proportion as found in skim milk and hence the two equations were added to give:

$$8CH_2O + C_8H_{12}N_2O_3 + 6O_2 \rightarrow 2C_5H_7NO_2 + 6CO_2 + 7H_2O$$

 $R.O. = 6/6 = 1.0$

The ammonia liberated in casein oxidation is used in the carbohydrate assimilation and there is no pH change. A well-aerated sludge skim milk mixture has a pH of about 7.0. According to the last equation, skim milk will yield $(2 \times 124)/(240 + 184)$ or 58.5 per cent of the weight as microbial tissue. This was somewhat greater than the 52 per cent observed in the continuous-flow experiments. The difference may have been the result of endogenous respiration.

Endogenous Respiration

After the cells are formed, there is a slow continuous oxygen requirement. The organisms oxidize their own tissue to supply energy of maintenance.

$$C_6H_7NO_2 + 5O_2 \rightarrow 5CO_2 + NH_3 + 2H_2O$$

 $R.Q. = 5/5 = 1.0$

If endogenous respiration is extensive there is no accumulation of sludge; if it is low, sludge accumulates. The equation shows that the oxidation of 1 mole of cells with a formula weight of 113 requires 160 weight-units of oxygen. In order to determine the amount of cells actually oxidized at a Q_{02} of 10, the following calculations were made⁵⁶:

22.4 liters
$$O_2 = 32 \text{ g } O_2$$

1 ml = 1.43 mg O_2
 Q_{O_2} of 10 = 14.3 mg $O_2/\text{g cell/hr}$

Calculated from the equation for the conversion to cell tissue:

$$14.3 \text{ mg } O_2 = 10.2 \text{ mg cell tissue}$$

Hence, 1 gram of cell tissue will oxidize 10.2 mg of cell per hour which is equivalent to a reduction of about 1 per cent.

An endogenous Q_{02} of 10 would thus be equal to a decrease of cell substance of 1 per cent/hr, and one of 5 would equal 0.5 per cent/hr. The rate of oxidation is related to the activity of the mixed organisms. The time required is independent of the amount and inversely proportional to the rate. Rates averaging 1.25 per cent/hr have been obtained in field trials at the Pennsylvania State University.⁶⁸

Implication of Data

With the information readily obtained in the laboratory, it becomes possible to predict the amount of sludge produced in the aeration process and the quantity of oxygen required to produce this sludge. It is also possible to determine the rate at which oxygen must be supplied in order to maintain aerobic conditions. In these studies endogenous respiration required only about $\frac{1}{10}$ of the oxygen needed for assimilation and the sludge was oxidized at a rate of about 1 per cent of its own weight per hour.

The following calculations show what happens to 1 lb of skim milk dissolved in 1000 pounds of water or 1000 ppm in the presence of 500 ppm sludge solids. The total theoretical amount of oxygen needed for complete oxidation is 1.214 lbs.

In the assimilation phase:	
Part of total O ₂ required	37.5%
Quantity O ₂ needed	0.453 lb
Time required	6.0 hr
Hourly utilization of O ₂	0.075 lb (av.)
Cells produced	0.5 lb
In the endogenous phase:	
Part of total O ₂ required	62.5%
Quantity O ₂ needed	0.761 lb
Time required to oxidize the 0.5 lb cells (500 ppm)	100 hr (approx.)
Hourly utilization of O ₂	0.007 lb

The tabulation shows that conditions may be established to avoid accumulation of sludge or microbial cells. If 2500 ppm sludge solids were carried in the aerator, 500 ppm would disappear in 20 hours by endogenous respiration. These cells could be settled and the clear liquid discarded. The addition of sufficient skim milk to give 1000 ppm waste-concentration would replace the cells destroyed by endogenous respiration. The process of fill-and-draw could be repeated indefinitely. Equations for maintaining a sludge balance were developed in field studies.⁶⁸

Storage and Oxidation

The application of improved experimental procedures has led to interesting observations when industrial wastes are treated by aerated sludge. Gellman and Heukelekian²² show that the rates of purification of seven different

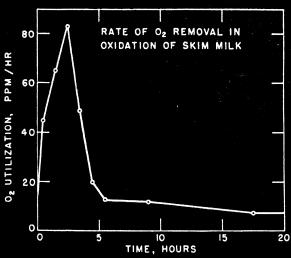


FIGURE 1-25. Hourly oxygen requirement by 1000 ppm milk solids in presence of 500 ppm sludge solids.

TABLE 1-13. EMPIRICAL COMPOSITION OF AERATED SLUDGE ORGANISMS

Component	Per Cent	Per Cent of Atomic Weight	Number of Atoms
\mathbf{C}_{i}	47.26	3.94	4.9A
H	5.69	5.65	7.0
N	11.27	0.81	1.0
O	27.0	1.69	2.1
Λsh	8.61	-	_
Total	99.83		

Empirical formula = C₅H₇NO₂

Empirical Composition of Sludge Cells

The oxidation of the organic constituents of a waste requires many intricate steps before the ultimate cell is produced. For the purpose of following the biological oxidation and determining oxygen demand, however, cell synthesis may be expressed in a relatively simple way. An empirical composition of the cells was established only after direct analyses were made of a well aerated sludge for oxygen, carbon, hydrogen, nitrogen, and ash. The amount of each element present in the sludge was calculated to a molar basis by dividing by its atomic weight. The resulting formula $C_5H_7NO_2$ (Table 1-13) is a gross oversimplification of the organized system of the microbial cells. The formula expresses the average proportions of these major atoms of the organic composition. Thus $C_5H_7NO_2$ has a "mole weight" of 113 and when ash was added the "weight" became 124 atom units.

Lactose Conversion to Sludge Cells

A portion of the carbohydrate was completely oxidized to produce energy for the conversion of the remainder to cell tissue or sludge solids. Since byproducts of incomplete oxidation were absent, the sugar was completely oxidized in this energy-yielding step, thus:

$$C_{12}H_{22}O_{11} \cdot H_2O + 12O_2 \rightarrow 12CO_2 + 12H_2O$$

which for convenience may be expressed:

$$CH_2O + O_2 \rightarrow CO_2 + H_2O$$

 $R.Q. = 1/1 = 1.0$

The production of cell tissue or sludge solids from the carbohydrate, using ammonia as a source of nitrogen, is as follows:

$$5CH_2O + NH_3 \rightarrow C_5H_7NO_2 + 3H_2O$$

This equation is balanced without oxygen utilization or carbon dioxide evolution. Manometric data showed that 37 per cent of the theoretical amount of the total oxygen required was used during assimilation; hence, only 3/8 or 37.5 per cent of the sugar was oxidized:

$$8CH_2O + 3O_2 + NH_3 \rightarrow C_5H_7NO_2 + 3CO_2 + 6H_2O$$

 $R.Q. = 3/3 = 1.0$

The experimental R.Q. of 1.04 agrees well with the theoretical value. The yields by weight were also consistent; 8CH₂O or 240 atom units yield C₅H₇NO₂ or 113 atom units equalling 124 atom units including ash. This is 52 per cent yield by weight, approximating that obtained in earlier experiments.

Casein Conversion to Sludge Cells

An empirical formula for casein was established from a direct determination of its major constituents. Phosphorus and sulfur were omitted as only about 0.8 per cent of each is found. Casein has a formula of $C_8H_{12}N_2O_3$ and a mole weight of 184. Complete oxidation of casein with the formation of ammonia may be written:

$$C_8H_{12}N_2O_3 + 8O_2 \rightarrow 8CO_2 + 2NH_3 + 3H_2O$$

 $R.Q. = 8/8 = 1.0$

Assimilation of casein into cell tissue, according to manometric results, may occur thus:

$$C_8H_{12}N_2O_3 + 3O_2 \rightarrow C_6H_7NO_2 + NH_3 + 3CO_2 + H_2O$$

 $R.O. = 3/3 = 1.0$

A N considered as a single atom.

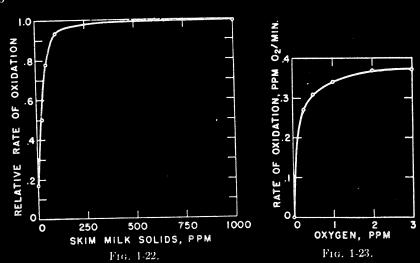


FIGURE 1-22. Relative rate of oxidation by 500 ppm aerated sludge as a function of skim milk solids. Values calculated from time required to reduce the oxygen to 0.5 ppm (see Figure 1-21). Highest rate with 1000 ppm substrate taken as unity.

FIGURE 1-23. Oxidation of 100 ppm skim milk solids as a function of oxygen concentration. Rate taken from Figure 1-22.

were obtained with a sludge containing 500 ppm cells. Increasing the sludge concentration would also decrease the time required for waste conversion.

These series of tests carried out in the Lingane cell were not agitated or aerated, and the endogenous cells removed oxygen only one-fifth as fast as the rapidly assimilating cells. Later studies made with vigorous agitation and aeration gave endogenous rates of respiration one-tenth or less than that of the actively growing cells.

Figure 1-23 shows the rate of oxidation of 100 ppm skim milk by 500 ppm cells as a function of oxygen concentration. The rates are taken from Figure 1-22. The rate of oxidation and hence oxygen removal dropped sharply in the region of an oxygen concentration of 0.35 to 0.5 ppm because of the lack of oxygen. Below this oxygen concentration anaerobic conditions tend to prevail. Absolute removal of oxygen at these solids concentrations was 0.35 ppm per min or 21 ppm per hr. The necessity of supplying oxygen at this rate becomes obvious in order to treat the waste aerobically.

Oxygen Utilization in Aerated Solutions

The rate and extent of oxidation of the waste were also determined by measuring the CO₂ evolved from a vigorously aerated sample by passing the spent air through barium hydroxide. This was possible since the volume of CO₂ evolved from this system was practically equal to the O₂ consumed in the Warburg respirometer. Under the conditions of this experiment,

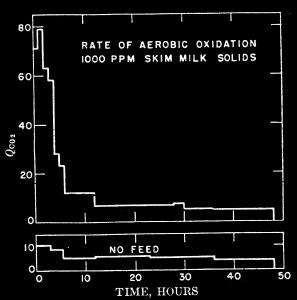


FIGURE 1-24. Rate of CO₂ formation per mg sludge by 410 ppm sludge in the presence and absence of 1000 ppm skim milk.

the rate of oxidation of milk solids proceeded at approximately 10 times that of endogenous respiration.⁵⁶

Figure 1-24 shows the results obtained when 500 ml of a mixture containing 410 ppm sludge solids and 1000 ppm milk solids was aerated over a period of 48 hours. The results are given in absolute units and are calculated to the original sludge weight. The Q_{CO_2} (ml CO₂ per grams sludge per hr) will equal the Q_{O_2} and may be converted to the weight of oxygen. (1 ml oxygen weighs 1.43 mg.)

It was shown repeatedly there was an immediate high rate of oxidation caused by assimilation. By the sixth to seventh hour this rate dropped and approximated the rate of the unfed sample, especially when the increased quantity of newly formed sludge was taken into consideration. The endogenous respiration dropped from 10 to 4 ml CO₂ per gram per hr in the unfed sample and to about 8 ml CO₂ per gram per hr in the fed sample. From these data the oxygen utilized was calculated. Figure 1-25 shows that 83 ppm O₂ per hr were required during the height of the assimilation reaction. This was about four times that needed when additional oxygen was not added, as in the polarographic study. This comparison illustrates the effect of vigorous agitation.

The oxygen uptake at 18 hours was only 7 ppm per hr. Air requirements in the latter stage were considerably less than those required in the first few hours.

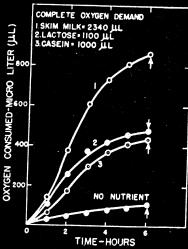


FIGURE 1-20. Oxidation of skim milk, lactose, and casein in respirometer.

TABLE 1-12. OXYGEN REQUIREMENTS AND UTILIZATION BY SUBSTRATES

Oxygen Utilization					
	Lactose Hydrate	Casein (8% water)	Skim Milk (air dry)		
Theory, mg/g	1060	1360	1110		
Theory, ml/g	742	952	780		
Sample used, mg	1.50	1.05	3.00		
Theory, ml/sample	1.1	1.0	2.3		
Observed, ml/sample	0.479	0.430	0.866		
Per cent	43	43	37		

lactose and the casein. The skim milk contained 36.3 per cent protein and 50.0 per cent lactose. Conversion to volume of oxygen from its weight is made by means of the well-known fact that 32 grams of oxygen occupies a volume of 22.4 liters at standard conditions.

Apparently the organic matter represented by 57 to 63 per cent of the original COD was assimilated by the sludge microorganisms, while the remainder was oxidized and used for energy required for the growth processes. These values are slightly higher than the 50 to 60 per cent reported earlier. Determinations of the respiratory quotient (R.Q.)* confirmed further that the substrate was assimilated or oxidized. The R.Q. found for the assimilation reaction was 1.00 and 1.03, values practically equal to the theoretical 0.96 and 1.00 for these substrates. Thus the conversion must be:

Milk wastes +
$$O_2 \xrightarrow{\text{microbes}} \text{sludge} + CO_2 + H_2O$$

Utilization of Oxygen

Experiments repeatedly showed a high utilization of oxygen during the assimilation phase, followed by a much slower rate of oxygen uptake during the endogenous phase.

A modified Lingane H-cell¹⁵⁹ was used in a polarographic method proposed by Hixson and Gaden⁵² for the determination of the relative rate of oxidation during assimilation and during the endogenous phase. A well-aerated sludge containing 500 ppm cell solids was used in these tests.⁵⁸ Varying amounts of waste were added and the air over the surface was displaced by nitrogen in order to assure uniform conditions. Figure 1-21 shows a relatively rapid and constant rate of skim milk oxidation by the 500 ppm sludge solids. The curves of oxygen depletion may be interpreted more satisfactorily when plotted as in Figures 1-22 and 1-23.

Rate of oxidation as a function of substrate concentration (Figure 1-22) was approximated from Figure 1-21 by determining the time required to reduce the oxygen tension to 0.5 ppm and considering the rapid rate of oxygen depletion as unity. The slowest rate of oxygen removal was obtained with unfed cells while the most rapid depletion was shown with 1000 ppm milk solids. The rate of oxidation and hence assimilation was fairly constant above a milk solids concentration of 100 ppm. Hence the time required to oxidize the waste would be directly proportional to the amount added in this range. Since 1000 ppm waste were oxidized in 6 hours in the Warburg studies, 500 ppm would take only 3 hours to be assimilated. These results

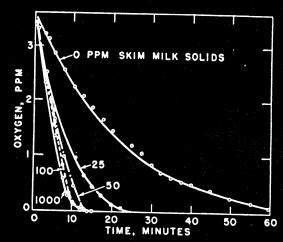


FIGURE 1-21. Removal of oxygen by 500 ppm aerobic organisms from nonaerated skim milk solutions.

^{*} Respiration quotient (R.Q.) = the ratio of the CO_2 produced to the O_2 used by the bacteria.

Aeration Studies

A synthetic dairy waste fed continuously through the 5-gallon fermenter at the slow rate of 1 to 2 volume changes per day showed a 40 to 50 per cent reduction in COD. Upon centrifugation of the effluent, the clear liquid showed a removal of 90 to 98 per cent of the original oxygen demand. This reduction resulted chiefly from the removal of carbohydrates, as shown on the balance sheet prepared from the influent and effluent solids (Table 1-11). Actually about one-half of the organic matter originally present was completely oxidized. The composition of the sludge or microbial cells in the effluent approximated 67 per cent protein, 11 per cent carbohydrate, and 8 per cent ash.

In other experiments, a fill-and-draw method was used in which one-fifth of the mixed solution served as seed or inoculum after 24 hours aeration. The starting seed contained 500 ppm sludge solids. Figure 1-19 shows the results when the simulated waste was added. As in the continuous flow experiment, the final COD reached a level between 50 to 60 per cent of that present in the original waste. Removing the solids gave about 90 per cent reduction in COD and much greater reduction in 5-day BOD. Under the

Table 1-9. Composition of Synthetic Waste Containing

OH TER CENT	DRIED	DKIM	MILK
Constituent			ppm
COD			1050
Organic solids			883
Lactose			508
Protein			369
Ash			81
Total solids			964

TABLE 1-10. ASH CONSTITUENTS OF TYPICAL SKIM MILK CONTAINING 10 PER CENT SOLIDS

(Kimin	per 1000	grams	nquid)	
Na ₂ ()	Mg()	Fe ₂ O ₂	$P_{z}O_{z}$	Cl

K ₂ O	CaO	Na ₂ ()	MgO	Fe ₂ O ₂	P_2O_4	Cl	SO:
1.88	1.51	0.75	0.18	0.01	1.82	1.07	0.29

Note: 10 ml of this skim milk diluted to 1 liter will give 1000 ppm.

Table 1-11. Organic Solids Balance in Aerobic Assimilation of Milk Waste by Sludge Microorganisms

((ĴB.	cu	a	æd	t	0	80	lio	н	basis	as	mg	١
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	Protein	Carbohydrate	Total
Skim milk waste	35	53	88
Effluent solids	34	7	41
Effluent solubles	· 1	2	3
Material destroyed	0	44	44

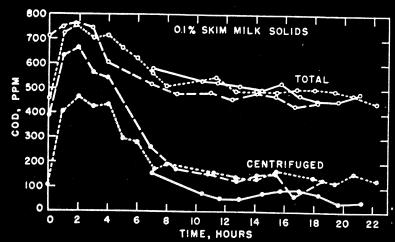


FIGURE 1-19. Changes in COD in fill-and-draw operations.

conditions of these tests, the changes were essentially completed in 6 to 8 hours, after an initial rise in COD caused by the added waste.

Manometric Studies

The oxidation of skim milk and of its major components, casein and lactose, was followed in a Warburg apparatus.⁵⁴ The temperature was maintained at 30°C (86°F), which was used throughout these studies. Manometric methods were feasible, as other workers have established that carbon dioxide is the only gas elaborated in the biological oxidation of sewage by sludge.^{13, 20, 151} The analytical data (Table 1-11) on solids balance further confirmed this fact.

The rate and extent of oxidation by 3 ml of well-aerated sludge containing 500 ppm solids were studied. Substrates were dissolved in phosphate buffer and added from the side arms. Figure 1-20 presents results obtained upon the addition of 3 mg skim milk (1000 ppm), 1.5 mg lactose (500 ppm), and 1.05 mg casein (350 ppm). The latter two were added in the proportion found in skim milk. At first there was a rapid demand for oxygen; in 6 hours this demand decreased to that of the sludge mixture alone containing no milk nutrient. The experiment was terminated at this time.

Determinations made for COD upon the supernatant solution showed that all added nutrients were removed. Calculations based upon the complete oxygen demand and upon the amount of oxygen utilized, however, failed to show complete oxidation of the substrate. Table 1-12 summarizes the data which showed that only 43 per cent of the COD of lactose and casein and 37 per cent of the skim milk were oxidized. The theoretical values for complete oxidation were calculated from the composition of the

be obtained by contacting waste and sludge at various loading levels for a predetermined period (usually 15 minutes) and computing absorption less oxidation and synthesis or by extrapolation of data plotted, as shown in Figure 1-12. The initial removal of BOD is of considerable importance in biological waste treatment, since it establishes minimum structural requirements. In domestic sewage treatment as much as 90 per cent BOD removal has been attained through the initial removal reaction.

In the second phase of the process BOD removal occurs concurrently with synthesis and oxidation and follows the log growth phase law of cell growth. Stored BOD removed on initial contact is subsequently oxidized and synthesized. A material balance relating BOD removal, sludge growth, and oxidation may be employed to determine oxygen requirement and excess sludge production for specific process operating conditions, as shown by Equations (4) and (11).

While endogenous respiration is presumed to occur under all ecological conditions, sludge is destroyed by oxidation when the organic loading is insufficient to support active growth. Extensive endogenous respiration will produce a sludge of low activity and reactive capacity.

A concept of evaluating the kinetics and equilibria of biological oxidation systems has been presented. It is believed this theory can provide practical assistance to the research worker in the design of experiments, to the designer in determining the economic effects of variations in process design, and to the operator in evaluating the influence of operating variables on plant performance.

1-3 PRINCIPLES OF BIOLOGICAL OXIDATION

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Removal of soluble organic matter from solution by microorganisms is dependent upon many factors. About 40 to 50 per cent of organic matter consists of carbon. To remove the organic matter, the carbon must be available to the organisms. In municipal sewage, there are sufficient essential elements to permit satisfactory activity of aerobic microorganisms. Deficiencies of nitrogen and phosphorus and possibly other elements often occur in certain types of industrial wastes. Sawyer discusses the importance of these in nutrition and synthesis. Wider recognition of such deficiencies and their correction are necessary, especially in the aerobic processes.

The investigations reported herein on the aerobic treatment of dairy wastes were summarized in a process report. Synthetic waste containing 0.1 per cent dried skim milk was used throughout these studies. This substrate gives a chemical oxygen demand (COD) by the rapid dichromate method of about 1050 ppm which approximates the 20-day BOD. (A rapid chemical method of determining oxygen demand is necessary because of the rapidity of the biological reactions in stabilizing the milk wastes.) Table 1-9 shows the composition of the waste. Many essential elements are in the ash fraction of the skim milk (Table 1-10). Dried skim milk contains about 8 per cent ash in addition to 37 per cent protein and 51 per cent lactose. Such a simulated waste satisfies the nitrogen demand of the aerobic organisms, as shown later in this paper.

Aeration processes are relatively rapid and devoid of acid and odor formation, if conducted in a satisfactory manner. Proper conditions of aeration were fairly easy to maintain in the aerobic fermenter used in these laboratory studies. Translating the results to pilot plant studies led to difficulties at first, but satisfactory engineering designs have been developed for industrial use by Kountz⁷⁴ and Eckenfelder. According to these workers, methods and information are now available for designing a proper and satisfactory waste disposal plant from laboratory data.

^{*} A laboratory of the Eastern Utilization Research Branch, Agricultural Research Service, U. S. Department of Agriculture.